Original Article

# Determination of radical scavenging activity of Creatine lysinate against methanol solutions of ABTS

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## ABSTRACT

Dietrary Creatine supplements can exert benefits against muscular dystrophic diseases, such as Duchenne muscular dystrophy, myophosphorylase deficiency (McArdle's disease), and fibromyalgia and can provide protection towards neurodegenerative disorders as Huntington, Parkinson and Amyotrophic lateral sclerosis. Creatine supplementation improves cognition and memory and can promote muscle strength. Creatine plays an important role in maintaining heart function in congestive heart failure. The current investigation's goal was to learn more about the ability of creatine lysinate to scavenge ABTS-radicals. Radiation scavenging activity [%], idex of inhibition (IC50), antioxidant power (1/IC50), and Trolox equivalent activity were calculated using the reduction in the absorbance at = 744 nm of the methanol solution of the ABTS-radical. Creatine lysinate (IC50 = 62.8 mM) exhibits an antiradical action, as shown by the experimental findings. According to the available data, creatine lysinate is less active than trolox (IC50 = 0.2 mM), which is because it has a greater IC50 value and less antioxidant capacity (1/IC50, = 0.016) than trolox (1/IC50, = 5). In current investigation was confirmed that ABTS-radical scavenging effect of Creatine lysinate is higher in comparison with Creatine monohydrate (IC50 = 100.98 mM), which was proven by the obtained higher Trolox equivalent antioxidant capacity TEAC = 0.003 of Creatine lysinate, compared with that of Creatine monohydrate (TEAC = 0.002).

**Keywords:** Creatine lysinate, ABTS, Trolox, Index of inhibition, Antitradical power

## Introduction

The overproduction of high reactive non-stable oxygen and nitrogen free radicals is the reason for occurring of oxidative stress. Another factor which contributes for the development of the oxidative stress is the downregulation in a defence mechanism of the endogenous antioxidant systems in organism. Endogenous free radicals are produced in mitochondria, endoplasmic reticulum and peroxisomes during the following biochemical processes in cells: oxidation of proatherogenic lipids VLDL and LDL, fatty acids, catecholamines, hemoglobin, pterins and thiols. In the process of oxidative phosphorylation, hyperoxides are synthesized in the mitochondria. Endothelial cells create superoxide radicals to control the vascular smooth muscle's capacity to contract [1].

Because brain neurons consume more oxygen, have more easily oxidizable unsaturated fatty acids in their membranes, and have fewer antioxidant defense systems than other neurons, they are more vulnerable to the effects of oxidative stress brought on by glutamate and beta-amyloid peptide. In Alzheimer's disease the increased oxidative stress is one of the initial neurological pathological changes as dendritic spine loss [2], synaptic [3] and mitochondrial dysfunction [4], and selectively occurs in areas of the brain, responsible for the regulation of memory functions.

Movement disorders affecting muscle function and coordination, and speech development are associated with deficiency syndromes characterized with muscle myopathies and cognitive

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problems due to low brain Creatine content [5]. For brain functioning, it is crucial that creatine be synthesized and transported throughout the central nervous system [6].

Creatine metabolism gene variability in children [7] is linked to deficient symptoms including muscle weakness, voluntary or involuntary movement [8], cognitive difficulties, or autistic disorder [7]. The production of creatine in the brain is crucial in cases of creatine-deficient disorders [9].

Numerous studies have found that taking a creatine supplement enhances learning and memory [10] and can increase muscular strength [11]. Creatine plays an important role in maintaining heart function in congestive heart failure [12] and in brain or heart ischemia-related damage [13]. It has been suggested that using creatine supplements will improve brain bioenergetics [14], skeletal muscle, bone and brain in aging population [15, 16]. Through its antioxidant effect, Creatine:

1. protects DNA and RNA from oxidative damage
2. preserves the integrity of mitochondria by preventing the formation of mitochondrial reactive oxygen species
3. helps maintain cellular levels of ATP, the marked reduction of which leads to the formation of radicals and tissue oxidative damage [17].

The therapeutic benefits of creatine supplements in the management of neuromuscular, muscular, neurological, and neurodegenerative disorders like Parkinson's disease are determined by their complex antioxidant capacity. Lou Gehrig's disease (amyotrophic lateral sclerosis) [18], Huntington [19]. Creatine increases muscle mass and strength in muscular dystrophic diseases [20], such as Duchenne muscular dystrophy [21], myophosphorylase deficiency (McArdle's disease) [22].

The prevention of disorders linked to oxidative stress is aided by dietary supplements [23]. The use of multi-ingredient dietary supplements is possible to increase cognitive function [24]. Food components with the characteristics to scavenge free radicals are frequently estimated using the ABTS-assay [25]. By evaluating the reduction in absorbance at = 744 nm, the current study sought to assess the radical-scavenging ability and anti-radical power of creatine lysinate against methanol solutions of the ABTS radical.

## Materials and Methods

### *Materials*

#### *1. Test compound: Creatine lysinate*

#### *2. Standard for antiradical activity*

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, N:51796 PMV 291913).

#### *3. Reagents with pharmacopoeial purity*

1) ABTS: diammonium salt of (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma Aldrich, N:SLBH 2992 V)
2) potassium persulfate (Sigma Aldrich, N:BCBL 7396 V).

#### *4. Solvents with pharmacopoeial purity*

1) methanol (99.9 %) (Sigma-Aldrich, N:SZBD 063 AV UN 1230).
2) distilled water.

### *In-vitro estimation of anti-radical activity of Creatine lysinate by application of ABTS assay*

#### *1. Preparation of stock solutions of Creatine lysinate*

Phosphate buffer with pH = 6.8 (0.1 g $KH_2PO_4$, 0.2 g $K_2HPO_4$, 0.85 g NaCl in 100.0 ml distilled water) was used for dissolving and dilution of measured quantity of 2.7732 g Creatine lysinate (M = 277.32) in volumetric flask of 50.0 ml for the preparation of the stock solution with concentration 200 mM (0.2 M).

#### *2. Preparation of working solutions of Creatine lysinate*

For the preparation of working solutions with final concentrations (2 mM ÷ 160 mM): 2 mM, 10 mM, 20 mM, 30 mM, 40 mM, 80 mM, 120 mM, 160 mM, the phosphate buffer pH = 6.8 was used for separately dilution to 100.0 ml of aliquot parts respectively of 0.1 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 4.0 ml, 6.0 ml, 8.0 ml from stock solution of 200 mM Creatine lysinate.

#### *3. Preparation of stock mixed solution of 7 mM ABTS and 2.45 mM potassium persulfate*

The stock mixed solution was created by combining equal aliquot portions of 100.0 ml of 2.45 mM potassium persulfate solution and 100.0 ml of 7 mM ABTS stock solution (M = 548.7 and 0.3841 g, respectively, in 100.0 ml of phosphate buffer pH 6.8). The final reagent was left in the dark for 17 hours.

#### *4. Preparation of working methanol solutions of Trolox for ABTS-assay*

Different aliquot portions were diluted with methanol to create solutions with concentrations ranging from 0.002 mM to 0.75 mM after dissolving and diluting 0.0125 g of Trolox (M = 252.294) in a 50.0 ml volumetric flask [26].

### *Method. ABTS – assay*

ABTS working solution was obtained by dilution of ABTS stock solution 1 : 10 v/v with methanol. Different aliquot parts of 5.0 ml of this solution were mixed separately respectively with aliquot parts of 5.0 ml of the working solutions of Trolox (0.002 mM ÷ 0.750 mM) or of 5.0 ml Creatine lysinate (2 mM, 10 mM, 20 mM, 30 mM. 40 mM, 80 mM, 120 mM, 160 mM, 200 mM) to obtain final concentrations of Trolox (0.001 mM ÷ 0.375 mM) and of Creatine lysinate (1 mM ÷ 100 mM): 1 mM, 5 mM, 10 mM, 15 mM, 20 mM, 40 mM, 60 mM, 80 mM, 100 mM. As a control a mixture of 5.0 ml ABTS working solution and 5.0 ml of methanol was used. After shaking the samples were incubated for 10 min. in the dark at 25 °C ÷ 27 °C. An UV-VIS spectrophotometer (Hewlett-Packard A Diode Array 8452) was used for the measurement after incubation of the absorbances of samples at λ = 744 nm. Methanol was used as a compensatory solvent.

## Results and Discussion

### *1. Spectra and absorbance of ABTS-radical*

**Figure 1** illustrates the spectra of ABTS methanol solutions at λ = 744 nm after 10 min. interaction with Creatine lysinate solutions (1 mM ÷ 100 mM). The absorbance of control is 0.99425.

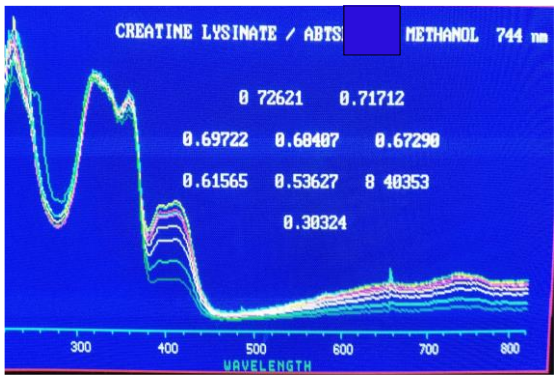


**Figure 1.** ABTS spectra after interaction with 1 mM ÷ 100 mM Creatine lysinate.

From the obrained ecperimental values of absorbances, the radical-scavenging activity [RSA, (%)] and the unbound ABTS-radical [R, (%)] for Creatine lysinate were calculated **(Table 1)**.

**Table 1. Absorbance, radical-binding activity [RSA] and unbound ABTS-radi-cal [R] after interaction with 1 mM ÷ 100 mM Creatine lysinate.**

| N: | C [mM] | A [AU] | RSA [%] | R [%] |
|---|---|---|---|---|
| 1. | 1 | 0.72621 | 26.96 | 73.04 |
| 2. | 5 | 0.71712 | 27.87 | 72.13 |
| 3. | 10 | 0.69722 | 29.87 | 70.13 |
| 4. | 15 | 0.68407 | 31.20 | 68.80 |
| 5. | 20 | 0.67290 | 32.32 | 67.68 |
| 6. | 40 | 0.61565 | 38.08 | 61.92 |
| 7. | 60 | 0.53627 | 46.06 | 53.94 |
| 8. | 80 | 0.40353 | 59.41 | 40.59 |
| 9. | 100 | 0.30324 | 65.50 | 30.50 |

The experimental data for the absorbance of ABTS methanol solution at = 744 nm were set against the corresponding concentrations in a linear regression analysis after 10 min. of interaction with creatine lysinate solutions (1 mM, 100 mM) **(Figure 2)**. The findings demonstrated that, at the concentration ranges under investigation, a drop in absorbances was seen as the concentration of the drug under investigation was raised. A coefficient of linear regression in the calibration curve that is greater than 0.97 was used to define linearity **(Figure 2)**.

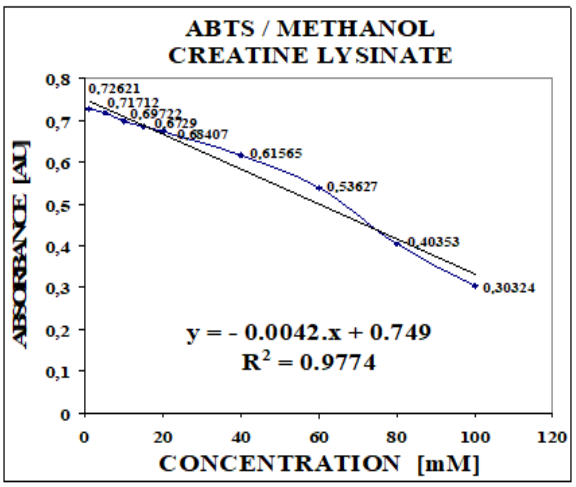


**Figure 2.** Absorbances of ABTS-radical after interaction with 1 mM ÷ 100 mM Creatine lysinate.

## 2. ABTS-radical scavenging effect

The radical-scavenging activity [RSA, (%)] for Creatine lysinate was calculated by the equation:

$$RSA[\%] = \frac{AABTScontrol - Asample}{AABTScontrol} \times 100 \qquad (1)$$

AABTS control: absorbance of the ABTS-radical solution before contact with the substance under investigation
Sample: the absorbance of the ABTS-radical solution after interacting with the target chemical

The data for ABTS-radical scavenging effect of Creatine lysinate (1 mM ÷ 100 mM) were subjected to a linear regression analysis. The calibration curve for the linear connection between the improved radical scavenging activity and the concentration increase from 1 mM to 100 mM is shown in **Figure 3**. Regression coefficient values greater than 0.97 demonstrate linearity.

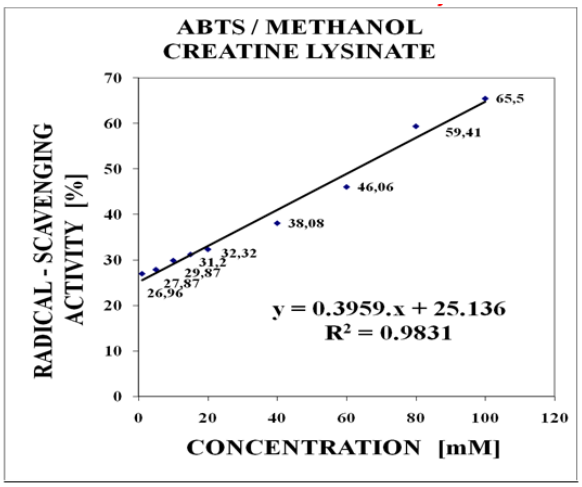


**Figure 3.** ABTS-radical scavenging activity of 1 mM ÷ 100 mM Creatine lysinate.

## 3. Unbound ABTS-radical

The values for the unbound ABTS-radical [R, %)] from Creatine lysinate (1 mM ÷ 100 mM) were calculated by the equation:

$$R[\%] = \frac{Asample}{AABTScontrol} \times 100 \qquad (2)$$

AABTS control: absorbance of the ABTS-radical solution before contact with the substance under investigation
Sample: the absorbance of the ABTS-radical solution after interacting with the target chemical

**Figure 4** shows calibration curve for linear relationship between the decreased s for not-scavenged ABTS- radical with the increase of concentration of Creatine lysinate from 1 mM to 100 mM.

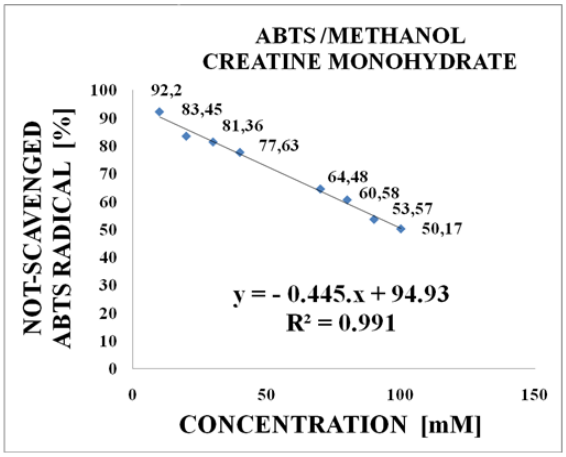


**Figure 4.** Not-scavenged ABTS-radical after interaction with 1 mM ÷ 100 mM Creatine lysinate.

## 4. Calculation of IC50 value (inhibitory concentration) and antioxidant power: $1/IC_{50}$...

Regression analysis was used as a technique. The IC50 value, which identifies the amount of antioxidant that reduces the number of radicals by 50%, was calculated using the derived regression equations for creatine lysinate.
For the calculation of $IC_{50}$ the following equatioms were used:

$$y = 163.2.v + 17.29 \quad \text{(Trolox) [26]} \qquad (3)$$

$$y = 0.3959.v + 25.136 \quad \text{(Creatine lysinate)} \qquad (4)$$

High radical-scavenging activity is associated with low $IC_{50}$ value, which defines that at lower concentration the compounds exert high antiradical effect.
In our previous srudy [26] it was confirmed that standard Trolox ($IC_{50}$ = 0.2 mM) exhibits the higher antioxidant effect than Creatine monohydrate ($IC_{50}$ = 100.98 mM; $1/IC_{50}$ = 0.01).
According to the current experimental findings, although creatine lysinate has an antiradical impact (IC50 = 62.8 mM; 1/IC50 = 0.016), it is less effective than trolox (IC50 = 0.2 mM) because it has a greater IC50 value and less antioxidant power (1/IC50, = 0.016) than trolox (1/IC50, = 5).

## 5. Calculation of Trolox equivalent antioxidant capacity

The Trolox equivalent antioxidant capacity (TEAC), which was determined as follows, was used to express the sample's ABTS radical scavenging activity:

$$TEAC = \frac{IC50Trolox}{IC50sample} \qquad (5)$$

The higher TEAC value means the higher ABTS radical-scavenging activity.
In our previous srudy [26] for Creatine monohydrate was calculated TEAC = 0.002. In current study for the ABTS-radical

scavenging activity of Creatibe lysinate, expressed as Trolox equivalent antioxidant capacity was obtained: TEAC = 0.003.

## Conclusion

According to the experimental findings, creatine lysinate has a stronger ABTS-radical scavenging activity than creatine monohydrate (IC50 = 100.98 mM), but a lesser scavenging impact than the standard drug Trolox (IC50 = 0.2 mM).

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Ethics statement: None

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